Table 2 Efficacy of donepezil hydrochloride and acetylcholine receptor (AchR) α7 polymorphism

	No.	Genotype W/W	W/M	M/M	χ^2-test
Responder (improved)	21	10	11	0	$P < 0.05$
Non-responder (not improved)	22	17	5	0	

Donepezil hydrochloride responders were more frequently heterozygous for a 2-base deletion in the AchR α7 gene ($P < 0.05$).

MARKERS FOR THE PREDICTION OF DRUG EFFICACY

With the marketing of donepezil hydrochloride, there is now a treatment for AD. However, responder and non-responder groups exist. Methods to differentiate these groups have also been investigated. As this drug is an acetylcholine esterase inhibitor, we focused our attention on acetylcholine receptor α7 genetic polymorphism, and found that donepezil hydrochloride responders were more frequently heterozygous for a 2-base deletion in the acetylcholine receptor α7 gene ($P < 0.05$, Table 2).[3] This polymorphism requires further analysis not only as a marker for the early detection of AD, but also as a diagnostic marker for predicting the drug efficacy.

CONCLUSION

We consider that the quantification of phosphorylated tau in the cerebrospinal fluid is currently the most reliable and useful biomarker for AD. The method involving the use of a touch panel computer can be recommended as a preliminary screening test. Acetylcholine receptor α7 genetic polymorphism may be a useful marker in the treatment with acetylcholine esterase inhibitors.

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ORIGINAL ARTICLE

Evaluation of a computerized test system to screen for mild cognitive impairment

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Abstract

Background: Mild cognitive impairment (MCI) refers to the clinical condition between normal aging and Alzheimer's disease (AD) and has a high probability of developing into AD. Early detection of MCI is important because early detection and appropriate follow-up treatment can prevent the disease from progressing. Therefore, MCI is an important candidate for screening and possible intervention.

Methods: We have developed a computerized screening test system to identify cognitive decline. This system consists of six tests (age and year-of-birth validity test, three-word memory test, time orientation test, first modified delayed-recall test, visual working memory test and second modified delayed-recall test). The scores obtained from three groups (MCI patients, AD patients and healthy control subjects) were analyzed to evaluate the sensitivity and specificity required for the screening of MCI.

Results: The system was well accepted by the patients. All of the test procedures were completed within 5 min. Significant group differences in all test results were found. The system has sensitivity and specificity values of 82% and 87%, respectively, when used as a screen for MCI.

Conclusion: The system is useful for the screening of cognitive disorders.

Key words: Alzheimer's disease, computer, dementia, mild cognitive impairment, screening.

INTRODUCTION

The population of aged persons is increasing along with the extension of the average span of human life. The number of aged persons over 65 years of age is now over 24 million, which accounted for 19% of all Japanese people in 2003. The prevalence rate of dementia was reported to be 7.5% of all people over 65 years of age.[1] This means that the number of persons with dementia is estimated to have reached more than two million in Japan. Therefore, preventing an increase in the number of persons who suffer from cognitive disorders is regarded as an urgent task. Nowadays, a lot of attention is focused on mild cognitive impairment (MCI).[2] MCI refers to the clinical condition between normal aging and Alzheimer's disease (AD) and has a high probability of developing into AD.[3] Early detection of MCI is important because early detection and appropriate follow-up treatment can prevent the disease from progressing.[4] Therefore, MCI is an important candidate for screening and possible intervention.[5,6] In the case of mass screening, the necessary requirements which a test method must fulfill are speed, objectiveness and unbiased results even if the examiner changes. Using a computerized cognitive test system yields some useful advantages; for example, it can provide quick, objective and precise results based on the same standard.[7] We have developed a computerized screening test system to identify cognitive decline. In this paper, we present a description of the system and the results obtained in our study.

SUBJECTS AND METHODS

Subjects

Fifty-one outpatients in the memory disorder and dementia clinics at Tottori University Hospital were enrolled for the study. They received neuropsychological tests as well as a neuroimaging examination and other medical checks. The diagnosis of dementia was made according to the *Diagnostic and Statistical Manual of Mental Disorders*, revised third edition (DSM-III-R)[8] criteria, and the diagnosis of AD was based on the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS–ADRDA)[9] criteria. The diagnosis of MCI was made according to the five criteria for amnestic mild cognitive impairment, which are: (i) subjective memory complaints; (ii) impaired memory function for age and education level; (iii) preserved general cognitive function; (vi) intact activities of daily living; and (v) absence of dementia, as previously reported.[3] Finally, 29 patients (eight men and 21 women; mean age of 78.1 ± 5.2 years) were diagnosed with senile dementia of the Alzheimer type (SDAT) and 22 patients (eight men and 14 women; mean age of 72.0 ± 9.6 years) were diagnosed with MCI. We recruited healthy control subjects from among the people who attended the Health Promotion event and from the patients' spouses who visited the hospital. Fifty-five persons (11 men and 44 women; mean age of 72.6 ± 7.3 years) accepted our invitation to participate in the study.

Computer system description

The computer program was developed with Microsoft Visual Basic 6.0 and runs under the Windows operating system on any IBM PC-compatible computer. The computer must have an audio output device because audio instructions are provided along with written instructions. The system has been designed for users who are mainly aged people. Most aged people can hardly operate a computer by using a keyboard and mouse. Therefore, we adopted a touch screen display as an input device so that aged people can operate the system easily. All interactions can be performed by touching the icon shown on the display without using a keyboard and mouse. Throughout the whole process, users are guided not only by text prompts, but also by voice instructions. In this study, we used a Hitachi PC1NH5 laptop computer (Hitachi, Tokyo, Japan) and a Totoku CV511PJ touch screen display (Totoku, Tokyo, Japan).

Details of test procedure

The system presents six tests as follows:

1. Age and year-of-birth validity test

The purpose of this test is to examine the subject's ability to recall his or her age and year of birth. The system will ask the subject for his or her age and year of birth, and the subject is then required to respond by touching the corresponding icons on the screen display. The system awards one point for the correct response.

2. Three-word memory test

The purpose of this test is to assess the immediate memory of the subject. The system will voice three words (for example, apple, swallow and car) and will immediately ask the subject what the words were. Then the system displays nine choice icons and requires the subject to select the three correct icons. The system awards a maximum of three points, one for each correct icon chosen. Before this test ends, the system informs the subject that the following modified delayed-recall test will query the subject for the three words again.

3. Time orientation test

For this test, the system presents four screens in turn, and asks the subject what year, month, date and day it is, respectively. The system awards a maximum of four points, one for each correct response.

4. First modified delayed-recall test

The purpose of this test is to assess the short-term memory of the subject. The system asks the subject to recall the same words voiced in Test 2. Nine choice icons are displayed as in Test 2, but the arrangement is different. The system awards a maximum of three points, one point for each correct answer.

5. Visual working memory test

The purpose of this test is to examine the visual working memory of the subject. Two types of figures are

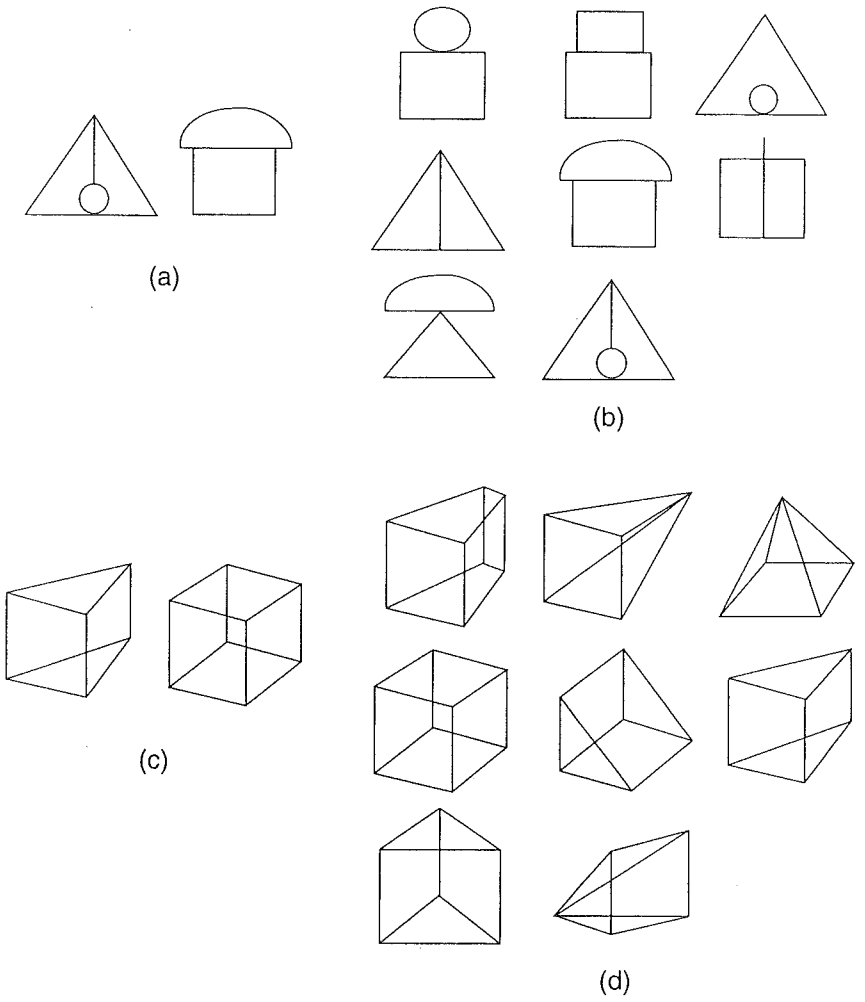

Figure 1 Figures used in the visual working memory test. (a) 2-D target stimuli; (b) 2-D figure choices; (c) 3-D target stimuli; and (d) 3-D figure choices.

used for this test: two-dimensional (2-D) and three-dimensional (3-D). Figure 1 shows the target figures and choices for this test. First, two 2-D target figures are presented for 10 s, after which the system displays eight possible choices of 2-D figures. The subject is then required to select the two previously displayed figures from among the eight choices. One point is awarded for each correct answer, for a maximum of two points. This process is then repeated with 3-D figures.

6. Second modified delayed-recall test

This test also assesses the short-term memory of the subject and is similar to the first delayed-recall test (Test 4, explained above). A maximum of three points are awarded, one for each correct response. For this test, the arrangement of the choice icons is different from that in Test 2 and Test 4.

All tests are usually completed within 5 min and the maximum number of points that can be awarded is 18 points.

Statistical analysis

Variations between the groups were first analyzed for significance by the Kruskal–Wallis test and then pairs of groups were directly compared using the Mann–Whitney test. Moreover, the differences between the scores obtained from three tests (three-word memory test; first and second modified delayed-recall tests) for each group were examined by the Friedman non-parametric test. These three statistical procedures were performed using statistical software (SPSS version 11 for Windows; SPSS Inc., Chicago, IL, USA). Receiver operating characteristic (ROC) analysis was performed to determine optimal cut-off points for the total score correspond-

Table 1 Demographic characteristics of samples and the results of individual tests for all three groups

	Control	MCI	SDAT
Sample size	55	22	29
Age (years)	72.6 ± 7.3	72 ± 9.6	78.1 ± 5.2
Sex (M/F)	11/44	8/14	8/21
Age and year-of-birth validity test	1 ± 0.00	0.89 ± 0.32	0.66 ± 0.48
Three-word memory test	2.96 ± 0.20	2.83 ± 0.38	2.66 ± 0.61
Time orientation test	3.86 ± 0.35	3.83 ± 0.38	1.69 ± 1.31
First delayed-recall test	2.94 ± 0.24	2.67 ± 0.49	1.76 ± 1.15
Visual working memory test for 2-D figures	1.88 ± 0.33	1.83 ± 0.38	0.97 ± 0.78
Visual working memory test for 3-D figures	1.80 ± 0.40	1.44 ± 0.51	1.31 ± 0.66
Second delayed-recall test	2.86 ± 0.35	2.44 ± 0.51	1.38 ± 0.98
Total score	17.31 ± 0.93	15.94 ± 1.26	10.41 ± 4.06

Results are expressed as: mean ± standard deviation. MCI, mild cognitive impairment; SDAT, senile dementia of the Alzheimer type.

Table 2 Results of the Kruskal–Wallis test showing significant group differences for all tests

	χ^2-value
Age and year-of-birth validity test	20.2**
Three-word memory test	9.39*
Time orientation test	59.3**
First delayed-recall test	35.4**
Visual working memory test for 2-D figures	37.3**
Visual working memory test for 3-D figures	15.7**
Second delayed-recall test	49.3**
Total score	65.0**

*$P < 0.01$, **$P < 0.001$.

ing to the screening between MCI and control subjects.

RESULTS

All subjects could understand how to interact with the computer and could complete the tests by themselves. Table 1 presents the demographic characteristics of samples and the results of individual tests from the three groups. At the group level, the SDAT group performed worse than the other two groups in each of the six tests, and the control group performed best in each of the six tasks. The results of the Kruskal–Wallis test are shown in Table 2 and significant group differences can be found for all tests. The results of the Mann–Whitney test in which the differences for all pairs of groups were compared are shown in Table 3. Significant differences between the control group and the SDAT group were found for all tests. Significant differences between the control group and the MCI group were found for the age and year-of-birth validity test, the visual working memory test for 3-D figures, the second delayed-recall test and the total score. Significant differences between the MCI group and SDAT group were found for the time orientation test, the visual working memory test for 2-D figures, the second delayed-recall test and the total score. The results of the Friedman nonparametric test are shown in Table 4. Significant differences were found in the SDAT and MCI groups at 99.9% level ($P < 0.001$) and 95% level ($P < 0.05$), respectively, and no significant differences were found in the control group. ROC analysis to identify the control and MCI yielded the best sensitivity and specificity values of 82% and 87%, respectively, with a cut-off point of 16.

DISCUSSION

The mini-mental state examination (MMSE)[10] is a widely recognized tool used for the detection of cognitive impairment, and in addition appears to be quite useful for examining patients with an increased risk of dementia (e.g. MCI patients).[11] When we developed the screening system, we referred to the tests used in the MMSE. However, these were originally designed for a method based on face-to-face interviews, so it was difficult to adopt all the tests for the computerized procedure. We paid attention to the tests of memory and the tests of visual working memory which are sensitive neuropsychological measures to detect early cognitive decline. Finally, we assembled the six tests explained above to form the computerized test system.

The mean value of the total score for all tests decreases in this order: control group, MCI group and SDAT group. In the validity test for age and year of

Table 3 Results of the Mann–Whitney test comparing the differences in all pairs of groups

	Control and MCI	Control and SDAT	MCI and SDAT
Age and year-of-birth validity test	*	**	NS
Three-word memory test	NS	**	NS
Time orientation test	NS	**	**
First delayed-recall test	NS	**	NS
Visual working memory test for 2-D figures	NS	**	**
Visual working memory test for 3-D figures	**	**	NS
Second delayed-recall test	**	**	**
Total score	**	**	**

*$P < 0.01$, **$P < 0.001$. MCI, mild cognitive impairment; NS, not significant; SDAT, senile dementia of the Alzheimer type.

Table 4 Results of the Friedman non-parametric test analyzing the differences in the scores from three tests for each group

	χ^2-value
Control	7
MCI	8.22*
SDAT	32.1**

*$P < 0.05$, **$P < 0.001$. MCI, mild cognitive impairment; SDAT, senile dementia of the Alzheimer type.

birth, all control subjects gave the correct answer. However, some MCI patients and one-third of the SDAT patients gave the wrong answer. A person who is unable to give his/her correct age and year of birth can be suspected to have MCI or SDAT. A combination of the three-word memory test and the subsequent delayed-recall test has been proposed as a useful test to screen for dementia.[12] In this study, there were no significant differences between the three-word memory test and the two subsequent modified delayed-recall tests in the control group. However, it is statistically significant that the score for the latter test was worse than that of the earlier test in the MCI and SDAT groups. This result is especially obvious in the SDAT group. It also suggests that these tests are useful for identifying cognitive decline. Visual working memory is also an important factor in the diagnosis of dementia.[13] In this study, we used two types of figures as target stimuli for the visual working memory test: 2-D and 3-D figures. In the case of 2-D figures, there were no statistical differences between the control and MCI groups. However, in the case of 3-D figures, statistical differences were found between the control group and MCI group. As for the MCI patients, they performed as well as the healthy control subjects in the visual working memory test for 2-D figures, but their performance was significantly impaired in the 3-D visual working memory test compared to the control subjects. In contrast, the visual working memory of the SDAT group for 2-D and 3-D figures was significantly impaired compared to the control group. Therefore, the visual working memory test also seems to be a useful indicator in identifying cognitive decline.

Conventional neuropsychological tests, such as the MMSE, involve dialogs between the interviewer and subject. However, it is difficult for a computer to understand what a subject is saying. Therefore, the system can only be designed to recognize a response when the user touches the choice button shown on the computer screen. Showing the answer choices is like giving a small hint and it does not require users to generate answers freely. In the delayed-recall test, presenting cues during the elicit retrieval phase improves the specificity of distinguishing individuals with dementia from unimpaired elderly people.[14] People who are able to answer the question with the help of a hint seem to be able to retain their cognitive ability and they usually get a high score. However, people who obtain a low test score even when hints are given are strongly suspected to be in cognitive decline. The selection of one answer from a range of choices seems to be adequate for the assessment of memory impairment and the method is easily adopted as a computerized procedure. However, computers are unsuitable for the assessment of dialogic ability, such as fluency, and this is a disadvantage of using computers.

We were able to identify MCI patients with an accuracy of 82% in this study, which is similar to the results presented by Darby *et al.*[15] Some other studies using biochemical examinations[16] and medical imaging equipment[17] have distinguished normal aged persons from those with dementia with high sensitivity

and a specificity of over 90%. However, such examinations are invasive or expensive. Therefore, we think that neuropsychological tests like ours are better for mass screening at the present time.

As conventional tests are based on a face-to-face interview, we often feel awkward when patients are unable to answer the questions correctly. The patients also seem to be embarrassed. In such cases, even though they are in the presence of a doctor, it seems that the patients' pride is hurt by showing their cognitive decline. But in the case of a computerized screening system, they do not feel uncomfortable when they cannot answer the question correctly. There appear to be some advantages and disadvantages in the methods of assessment using human interviewers and using computers. Human interviewers can respond flexibly according to the condition of the patient. However, their prescribed treatment is likely to be biased according to their experience or knowledge, and there is a risk that the assessment criteria could vary from tester to tester.[18] In contrast, the computerized testing system is good at extracting the required information based on the same standards without the above-mentioned problems. Standardization is required for screening tests to be consistent in assessment and for their widespread use. It has been pointed out that a useful dementia screening system should be fast, simple and accurate.[19] Our computerized test system can provide quick, objective and precise results based on the same standard. Although computerized testing cannot replace a human interviewer in all cases, this system is useful for the screening of cognitive disorders.

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A novel presenilin 1 mutation (Y154N) in a patient with early onset Alzheimer's disease with spastic paraparesis

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Abstract

Early onset familial Alzheimer's disease with spastic paraparesis (FAD-SP) has been associated with mutations of the presenilin 1 gene (*PSEN1*). We report a pedigree of FAD-SP due to a novel missense mutation of *PSEN1* (Y154N). The symptoms of the proband were characterized by presenile dementia in her 40s, preceded by spastic paraparesis in her 30s, whereas the mother of the proband presented with spastic paraparesis in her 40s, followed by symptoms of dementia in her mid 60s. The mutation was found only in the proband, and not in a normal family member, normal Japanese control subjects, patients with sporadic Alzheimer's disease or patients with familial spastic paraparesis without dementia. Thus, Y154N is a novel *PSEN1* mutation responsible for FAD-SP of Japanese origin.

Keywords: Presenilin 1; Alzheimer's disease; Spastic paraparesis

Alzheimer's disease (AD) is the most important neurodegenerative disorder leading to dementia. Familial Alzheimer's (FAD), especially the early onset (presenile) type, is inherited in an autosomal dominant fashion. The etiology of FAD is known as mutations in the amyloid precursor protein gene (*APP*) on chromosome 21, the presenilin 1 gene (*PSEN1*) on chromosome 14, and the presenilin 2 gene (*PSEN2*) on chromosome 1. Over 130 mutations of *PSEN1* have been found in FAD pedigrees all over the world (the detailed mutations of *PSEN1* are listed in the AD mutation database: http://molgen-www.uia.ac.be/ADMutations/default.cfm?MT=0&ML=0&Page=Home). Furthermore, *PSEN1* mutations have been found in several pedigrees with alternative clinical phenotypes of frontotemporal dementia and spastic paraparesis with dementia (or FAD with spastic paraparesis; FAD-SP). A deletion mutation (DeltaI83/M84) [5,15], an insertion mutation (InsFI) [12], seven-point mutations (F237I [14], V261F [12], R278T [8], R278K [1], E280G [11], P284L [17], and P436Q [5]) and four independent mutations resulting in deletion of exon 9 (Delta 9) [3–5,13] have been identified in the pedigrees of FAD-SP.

The most characteristic pathological feature of FAD-SP has been reported to be the so-called "cotton wool plaques", which are distinct from classical "senile plaques" [3–5,13,17]. However, the pathomechanisms for the formation of "cotton wool plaques" remain unclear.

Herein, we report a pedigree characterized by spastic paraparesis with dementia bearing a novel *PSEN1* mutation (Y154N).

A 47-year-old Japanese woman (proband, II-4) was admitted to our hospital complaining of progressive gait disturbance followed by gradual cognitive decline. Her first neurological symptom was gait disturbance noticed at the age of 37. At age 42, her family members noticed her first

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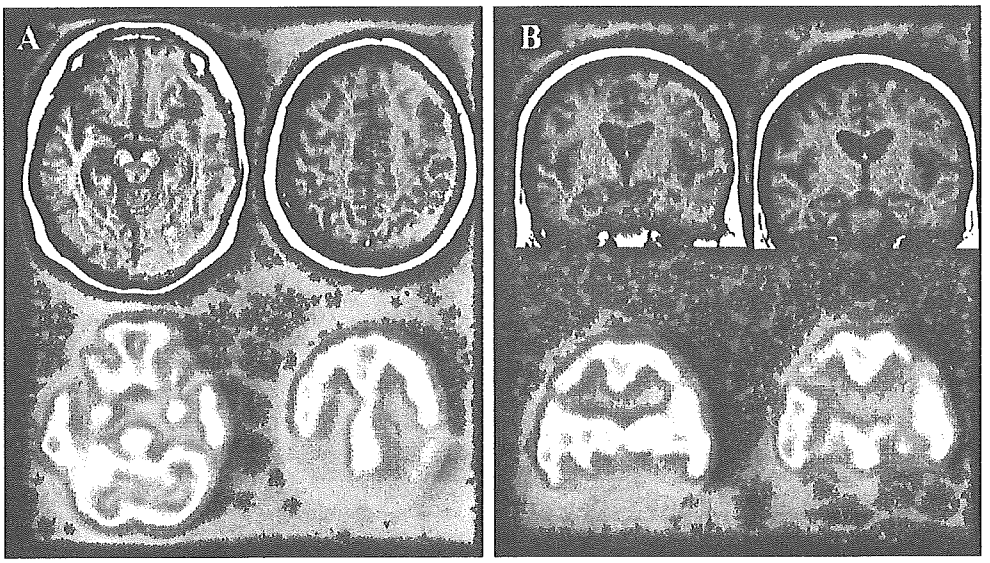

Fig. 1. MRI (T1 weighted image) and SPECT (^{99m}Tc-ECD) findings. (A) Axial T1 weighted images show apparent occipito-parietal atrophy and mild atrophy in internal portion of temporal lobes. Axial SPECT images show marked hypoperfusion in bilateral occipito-parietal areas and internal portion of temporal areas. (B) Coronal images show slight atrophy in bilateral hippocampal areas, whereas coronal SPECT images show apparent hypoperfusion in the internal portion of temporal areas including bilateral hippocampal areas.

amnesic symptoms. She had gradually progressive difficulties with her household chores. She also exhibited a mild slurring dysarthria. Neurological examination on admission at age 47 revealed pyramidal signs in her limbs consistent with spastic paraparesis. Deep tendon reflexes were hyperreactive in all limbs, and muscle tone was markedly spastic in her lower limbs. Pathological reflexes were detected in all limbs. She also had brisk jaw jerk. She showed marked spastic gait, predominantly in her left leg. She also exhibited apparent disorientation and prominent memory disturbance. Scores on the Mini-Mental State Examination and the WAIS were 16/30 and IQ 68, respectively. Levels of CSF amyloid β (Aβ) 42, total Tau protein (hTAU) and phosphorylated Tau protein (pTAU) were 373.4 pg/ml (non-AD controls of our department (nAD) were (n = 27); 1005 ± 248.1), 698.7 pg/ml (nAD (n = 23); 266.1 ± 191.9), 112.0 pg/ml (nAD (n = 23); 31.5 ± 32.8), respectively. The levels of Aβ42, hTAU and pTAU were measured using commercial kits (Innogenetics, Gent, Belgium). The ratios of hTau/Aβ42 and pTau/Aβ42 were 1.84 (nAD (n = 23); 0.31 ± 0.31) and 0.34 (nAD (n = 27); 0.049 ± 32.8), respectively. These results are consistent with previous reports that elevated ratios of hTau/Aβ42 and pTau/Aβ42 are useful CSF biomarkers for AD [6,9].

Cranial MRI showed mild cerebral atrophy in her temporal and parietal lobes (Fig. 1). ^{99m}Tc-ECD SPECT showed hypometabolism in the temporo-parietal and the internal portion of temporal areas (Fig. 1). According to these clinical data, we diagnosed our patient as having AD with spastic paraparesis.

Her family history indicated that the mother of the proband presented in her 40s with progressive gait disturbance and died at age 69. Although her family noticed that she had shown abnormal behavior and cognitive dysfunction consistent with having dementia 2 years before death, she had no episodes indicating her having dementia for at least 10 years after the onset of gait disturbance. Although the clinical manifestations were different between the proband and the mother, we assume that they are in a pedigree of FAD-SP.

After obtaining informed consent, DNA samples were extracted from peripheral blood leukocytes of the proband and her non-symptomatic elder sister. The coding regions and adjacent 5′ and 3′ intron sequences of *APP*, *PSEN1*, and *PSEN2* were screened by PCR-single strand polymorphism analysis (PCR-SSCP) and sequence analysis as previously described [18].

PCR-SSCP demonstrated an abnormal migration pattern in *PSEN1* exon 5 of the proband sample (data not shown), and sequence analysis identified a TAT to AAT substitution at codon 154 of *PSEN1* resulting in an amino acid substitution of tyrosine with asparagine (Y154N) (Fig. 2).

For screening of the Y154N mutation, PCR-restriction endonuclease length polymorphism analysis (PCR-RLFP) using a mismatch primer pair was used. In brief, DNA samples were amplified using a Hot-start PCR kit (TaKaRa, Shiga, Japan) and a primer pair; forward: 5′-GAATCTATACCCCATTCACAGAAGA-3′ and reverse: 5′-TCATGCTCACCTTATAGCACCTGTATTGAT-3′ (underline: mismatch position), followed by PCR-RLFP using *Hin*fI. The A allele of the Y154N mutation gains an artificial *Hin*fI site. In the PCR-RLFP using *Hin*fI, Y154N was detected only in the proband, and not in her non-symptomatic elder sister, 103 healthy controls, 15 patients with early-onset Alzheimer's disease (AD) without spastic paraparesis, 50 patients with late-onset AD and 7 independent patients with

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Elevated interleukin-6 levels in cerebrospinal fluid of vascular dementia patients

Wada-Isoe K, Wakutani Y, Urakami K, Nakashima K. Elevated interleukin-6 levels in cerebrospinal fluid of vascular dementia patients. Acta Neurol Scand 2004 DOI: 10.1111/j.1600-0404.2004.00286.x

Objectives – To investigate a possible implication of inflammatory processes in the development of dementia in cerebrovascular disease. *Patients and methods* – We examined the levels of interleukin-6 (IL-6) in the cerebrospinal fluid (CSF) of patients with Alzheimer's disease (AD) ($n = 26$), ischemic cerebrovascular disease without dementia (CVD) ($n = 11$), vascular dementia (VD) ($n = 11$), and other neurological disorders ($n = 21$) using sensitive enzyme-linked immunosorbent assay. *Results* – The CSF concentrations of IL-6 were significantly elevated in patients with VD compared with those of patients with AD or CVD. *Conclusion* – The CSF IL-6 levels are increased in patients with VD, suggesting that inflammatory mechanisms may be involved in the development of cognitive decline in some patients with cerebrovascular disease. CSF IL-6 may be a biological marker for dementia in cerebrovascular disease.

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Key words: vascular dementia; Alzheimer's disease; cerebrovascular disease; interleukin-6; cytokines; tau protein; cerebrospinal fluid; biological marker

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Vascular dementia (VD) is a common cause of dementia in Japan (1). However, the mechanisms of clinical cognitive deterioration in patients with cerebral ischemia are not completely understood. Several recent studies have provided insight into the possible role of inflammatory processes in the development of brain ischemia and multi-infarct cognitive impairment, as demonstrated by the accumulation of inflammatory cells and mediators in the ischemic brain (2–5). Vila et al. (6) reported that interleukin-6 (IL-6) participates in the acute-phase response that follows cerebral ischemia and that an association exists between high levels of IL-6 and early neurological worsening. A case–control genetic study reported a positive association of the -174 G/C IL-6 gene polymorphism and the risk of multi-infarct dementia (7). These data led the hypothesis that inflammatory mechanisms play a crucial role in the pathogenesis of the development of dementia in cerebrovascular disease. Cerebrospinal fluid (CSF) levels of IL-6 are elevated in central nervous system (CNS) infections and non-infectious CNS inflammatory diseases, indicating that levels of IL-6 in the CSF reflect the inflammatory processes (8–10). Little has been reported about the IL-6 levels in the CSF of patients with VD. Previous studies reported that these levels did not differ from those of controls (11–13). However, some patients with VD in the previous study investigated by Yamada et al. (13) showed high CSF concentrations of IL-6. In order to clarify the association of inflammatory mechanisms with VD, we examined the CSF levels of IL-6 in patients with VD as well as in patients with Alzheimer's disease (AD) and ischemic cerebrovascular disease without dementia (CVD).

Subjects and methods

The subjects were 26 patients with AD (mean age ± SD 66.8 ± 8.2 years), 11 patients with CVD (70.0 ± 6.2 years), 11 patients with VD (74.5 ± 4.5 years), and 21 patients with other neurological disease (OND) (68.4 ± 5.8 years). Assessments of these patients included a carefully examined medical history, physiological examination, drug inventory, neurological examination, comprehensive cognitive evaluation with the use of the Functional Assessment Staging of Alzheimer's disease (FAST staging), the Mini-Mental State Examination (MMSE), neuroimaging assessments of CT scan or MRI and single photon emission computed tomography of the head, and routine laboratory tests, such as blood analysis and biological

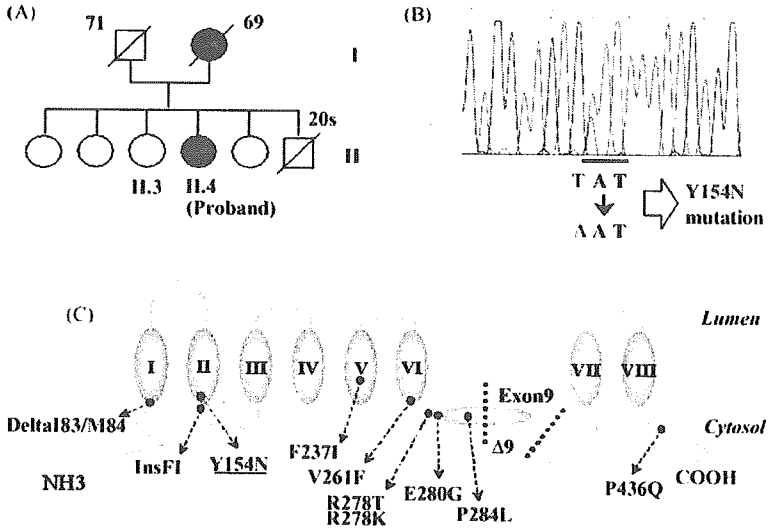

Fig. 2. (A) Pedigree of the family with Y154N *PSEN1* mutation. (B) Sequence diagram of *PSEN1* exon 5. Underlining indicates the heterozygous mutation from the T to A transition, resulting in a change from tyrosine (T) (TAT) to asparagine (N) (AAT) at codon 154 of *PSEN1*. (C) Schematic representation of *PSEN1* mutations for FAD-SP.

familial spastic paraparesis without dementia, indicating that the Y154N is specific to the proband.

Although several other polymorphic migration patterns in *APP*, *PSEN1*, and *PSN2* were detected by PCR-SSCP analysis, sequence analysis revealed that these polymorphic bands reflected the existence of single nucleotide polymorphisms (SNPs) listed in the NCBI SNPs database. APOE genotype of the proband was ε3/3.

We report here a novel PSN1 mutation (Y154N) presenting as FAD-SP. The Y154N mutation is in the cytosol side of predicted transmembrane (TM) domain 2 (Fig. 2) and takes place at the same residue as a previously found mutation (Y154C), which clinically presents as FAD without spastic paraparesis [7].

It has been reported that several *PSEN1* mutations are clinically associated with FAD-SP and cause accumulation of noncongophilic Aβ-positive "cotton wool" plaques in brain parenchyma [3–5,13,15,17]. Pathological analyses have demonstrated that "cotton wool plaques" are homogeneously positive for Aβ42 and negative for Aβ40, whereas amyloid core-like structures are positive for Aβ40 [3,4,15,17]. The deposition of Tau as neurofibrillary tangles is common, but variable in patients with FAD-SP [3,4,13,15,17]. In the proband with the Y154N mutation, the coexistence of Aβ and tau pathology was predicted by the results of CSF analysis, showing a decrement of Aβ42 and elevation of total and phosphorylated tau protein.

In cellular models bearing *PSEN1* mutations for FAD-SP (particularly in the Delta 9 mutation), a marked increase of Aβ42 production has been observed [2,10,16]. However, it has been reported that overproduction of Aβ42 in cellular models does not necessarily correspond to the clinical phenotype. Further, clinical manifestations (age of onset, initial symptom (e.g. dementia or paraparesis) and degree of dementia and spastic paraparesis) have been reported to be variable with the affected family members bearing the identical *PSEN1* mutation [1,3,13]. Assuming that the mother of the proband had the Y154N mutation, the age at onset of gait disturbance and cognitive decline, and the clinical course in this pedigree show generational differences. Based on this evidence, alternative cofactor(s) that can influence the clinical manifestations and/or the formation of "cotton wool" plaques in pedigrees of FAD-SP remain to be clarified.

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examination. Patients who satisfied the Diagnostic and Statistical Manual of Mental Disorders, third edition-revised (DSM-III-R) (14) and the diagnostic criteria of the National Institute of Neurological and Communicative Disorders Association (NINCDS-ADRDA) (15) and those scoring 4 points or less on Hachinski's ischemic score (16) were diagnosed as having AD. Patients who satisfied the DSM-III-R and the ADDTC criteria for ischemic VD (17) and those scoring 7 points or more on Hachinski's ischemic score were diagnosed as having VD. All the patients with VD showed stepwise deterioration of cognitive function and one or more infarcts outside the cerebellum detected by neuroimaging. The CVD group was defined as patients who had a history of stroke episode and with CT scan or MRI findings of infarcts without dementia. OND patients consisted of seven patients with Parkinson's disease (PD), four patients with amyotrophic lateral sclerosis (ALS), four patients with spinocerebellar degeneration, two patients with peripheral neuropathy, two patients with tension-type headache, one patient with myopathy and one patient with essential tremor. OND patients did not show any cognitive impairment. After informed consent from patients or their families, CSF was collected by lumbar puncture. CSF samples were stored at −80°C until assay. Collections of CSF from the patients with CVD or VD were performed during the chronic phase of the diseases when the progression of neurological deterioration was no longer observed. CSF IL-6 levels were determined in duplicate, using commercially available enzyme-linked immunosorbent assay (ELISA) kit (Quantikine; R&D Systems, Inc, Minneapolis, MN, USA). CSF total tau protein levels were measured using ELISA kit (Innogenetics, Gent, Belgium). Statistical significance was analyzed by one-way ANOVA, followed by *post hoc* tests. Correlation was analyzed by Spearman rank correlation test.

Results

As shown in Fig. 1, the concentrations of IL-6 in the CSF of VD patients were 5.67 ± 1.7 pg/ml (mean ± SE); those of patients with AD were 2.53 ± 0.87 pg/ml; those of patients with CVD were 2.15 ± 0.38 pg/ml; and those of patients with OND were 3.15 ± 0.67 pg/ml. Significantly elevated levels of IL-6 were found in the CSF of patients with VD compared with those in the CSF of patients with AD, CVD, and OND. There was no significant difference in CSF IL-6 levels between AD patients and CVD and OND patients. There were not a correlation between CSF IL-6 levels and

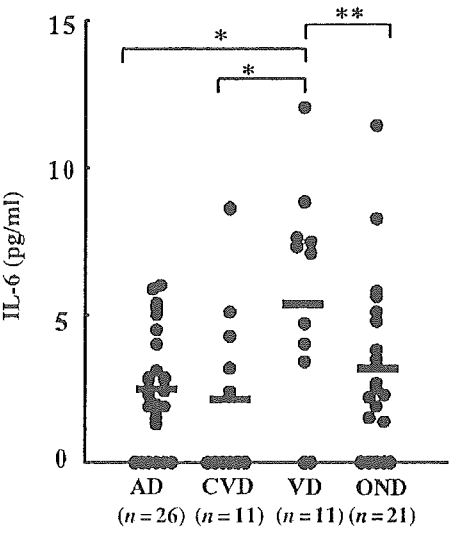

Figure 1. Interleukin-6 levels in the cerebrospinal fluid of the patients with Alzheimer's disease, ischemic cerebrovascular disease without dementia, vascular dementia, and other neurological disease. The horizontal bar indicates the mean level. Statistical differences were calculated using one-way ANOVA followed by a *post hoc* test; $*P < 0.01$, $**P < 0.05$.

Table 1 Total tau levels in cerebrospinal fluid

Disease	Mean ± SE (pg/ml)
AD	236.0 ± 17.2
CVD	115.0 ± 39.3
VD	116.8 ± 28.1
OND	126.4 ± 16.4

AD, Alzheimer's disease; CVD, cerebrovascular disease without dementia; VD, vascular dementia; OND, other neurological disorders.

MMSE scores in VD patients (data not shown). The levels of total tau protein in CSF were shown in Table 1. Significantly elevated levels of tau protein were found in the CSF of patients with AD compared with those in the CSF of patients with VD ($P < 0.01$). There were not significant differences between CSF tau levels in VD and those in CVD or OND.

Discussion

Interleukin-6 was described initially by Hirano (18) as a B cell differentiation factor usually derived from T cells and it can also be produced by astrocytes and microglia in the CNS (9, 19, 20). CSF levels of IL-6 were examined in patients with infectious or non-infectious inflammatory diseases of the CNS. CSF levels of IL-6 were also examined in patients with neurodegenerative disorders. In particular, controversial results ranging from no changed (21, 22), to increased (23) or decreased

(13) levels of IL-6 in the CSF have been reported in AD patients. Differences in sample size, selection of patients with AD and control subjects, or experimental procedures may account for these varying results. Our results show that there are no significant differences in the CSF levels of IL-6 between patients with AD and patients with CVD or OND. The OND group included patients with PD and ALS, in which higher levels of CSF IL-6 have been reported (23, 24). Indeed, the patients with the two highest levels in OND group in our study were a PD patient and an ALS patient, but CSF IL-6 levels were not altered in other patients with ALS or PD. We conclude that the levels of IL-6 are not altered in patients with AD, and that CSF IL-6 may not be a biological marker for the diagnosis of AD. On the contrary, we obtained significantly higher CSF levels of IL-6 in patients with VD, but not in patients with CVD who did not have dementia. However, previous reports indicated that CSF levels of IL-6 in VD patients were not significantly elevated (11, 12). It has been reported that patients with VD are heterogeneous and diagnosis criteria for VD are not interchangeable (25). Selection of patients may account for these differences. Not all the patients with VD showed higher levels of IL-6 in CSF in this study, but we used the DSM-III-R and ADTTC criteria for diagnosis of VD and employed probable cases in this study and these cases also showed significant lower levels of total tau protein in CSF compared with AD patients, suggesting that clinical diagnosis of VD patients was sufficient to segregate AD patients from VD cases. Recent reports indicated inflammatory process might be involved in cerebrovascular disease. Higher baseline levels of CSF IL-6 were shown to be related to early neurologic worsening in ischemic stroke, independent of the initial size topography or mechanism of ischemic infarction (6, 26). A genetic association of IL-6 polymorphism with multi-infarct dementia (7) and activation of the micoglia in Binswanger's disease, a form of VD has been shown (3). The increased intrathecal production of granulocyte-macrophage colony stimulating factor (GM-CSF), a cytokine that stimulates microglial cell growth and has inflammatory properties, has been found in patients with VD (4). Taken together with our result, the inflammatory activations in the CNS might be associated with some part of VD patients and measurement of CSF IL-6 might provide a clue to differential diagnosis of dementia. Our study is a cross-sectional design, further studies using a longitudinal design with large samples are necessary to support these results.

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SHORT REPORT

Novel amyloid precursor protein gene missense mutation (D678N) in probable familial Alzheimer's disease

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Objective: To describe a novel missense mutation, Asp678Asn (D678N), in the amyloid precursor protein (APP) gene in a Japanese pedigree of probable familial Alzheimer's disease (FAD).

Subject: The proband was a women of 72. Symptoms of dementia that fulfilled the criteria for probable Alzheimer's disease appeared at about 60 years of age, and slowly worsened over more than 10 years without evident cerebrovascular complications, either clinically or neuroradiologically.

Methods: Polymerase chain reaction single strand conformational polymorphism (PCR-SSCP) analysis followed by sequence analysis was used to examine genomic DNA of the proband for mutations in the APP gene exons 16 and 17.

Results: Analysis of the APP exon 16 in the proband showed a GAC to AAC nucleotide substitution in codon 678 of the APP gene, causing an amino acid substitution of Asp to Asn (D678N). Heterozygosity of the APP D678N mutation was found in the proband and in the demented elder sister.

Conclusions: The production and accumulation of mutated Abeta (Asn7-Abeta) or the misfunction of D678N mutant APP may have pathogenic properties for the development of Alzheimer's disease in this pedigree.

Mutations in three causative genes—amyloid precursor protein (APP),[1] presenilin 1 (PS1), and presenilin 2 (PS2),[2 3]—have been implicated in the pathogenesis of familial Alzheimer's disease (FAD). Mutations in the APP gene located close to the β- and γ-secretase cleavage sites lead to early onset FAD, whereas the clinicopathological representations of genes within the sequences coding for amyloid β peptide (Abeta) sequence are variable—that is, hereditary amyloid angiopathy with cerebral haemorrhage (HCHWA-D, Dutch mutation; E693Q, Italian mutation; E693K),[4] Alzheimer's disease with repeated stroke (Flemish mutation; A692G),[5] dementia with severe amyloid angiopathy (Iowa mutation; D694N), or Alzheimer's disease without stroke (Arctic mutation; E693G).[6 7] Here, we describe a novel APP mutation (D678N) in a Japanese-Tottori FAD (Jp-T) pedigree. The D678 mutation replaces the aspartate (Asp; D)7 of Abeta with asparagine (Asn; N) and was clinically linked to clinically diagnosed FAD without signs of vascular involvement.

METHODS

Patients

The Jp-T pedigree consisted of three demented patients and nine siblings (fig 1A). The proband (II-12) of this pedigree (fig 1A) was admitted to our hospital at 65 years of age, at which time he had been experiencing amnesia, disorientation, loss of interest in previous household activities, and occasional aggressive behaviour for over six years, without stroke-like episodes. Initial examination revealed moderate intellectual deterioration, severe memory impairment without focal neurological signs and parkinsonism. The mini-mental state examination (MMSE) score at this time was 9/30. Magnetic resonance imaging (MRI) showed mild cortical atrophy of the bilateral medial temporal lobes and parietal lobes. On ^{132}I-IMP SPECT there was marked hypoperfusion in the temporal and parietal cortices and normal blood flow in the cerebellum, motor cortex, and basal ganglia. The total tau protein and Abeta42 levels in the spinal fluid were 738 pg/ml (normal <250 pg/ml) and 295 pg/ml (normal >700), respectively, consistent with the characteristic findings of Alzheimer's disease. The dementia syndrome gradually progressed over the next seven years. 1

Serial MRI at age 72 showed marked cortical atrophy including the hippocampal region bilaterally, without focal cerebral infarction or haemorrhagic lesions (fig 1B).

At the time of writing, her dementia symptoms had progressed to such a degree that she required total assistance in dressing, hygiene, and food intake. Her communication skills had severely deteriorated and dyskinetic movements in face and head without myoclonus were observed. 2

The proband's elder sister (II-10) had been experiencing amnesic symptoms that appeared when she was 60. Five years after the onset of her symptoms, she was unable to recognise her family. Over the next 15 years, she showed slowly progressive cognitive decline without stroke-like episodes. At age 75, she was severely demented, bed ridden, and fully dependent for personal care, and oral dyskinesia was observed. MRI at the age of 77 showed severe cerebral atrophy with mild periventricular hyperintensity lesions in the T2 weighted images but without focal cerebral infarction or haemorrhagic lesions (fig 1, C). The mother had no symptoms of dementia before she died at age 85. The father died at age 64 from an accident and it is unclear whether he was demented. It was reported that the eldest brother (II.1), who died at age 69, had an almost identical medical history of dementia beginning in his late 50s. The fourth to ninth and the 11th siblings all died aged less than 50 years.

Genetic analysis

After obtaining informed consent (from relatives where appropriate), genomic DNA was isolated from peripheral white blood cells of three family members: patient 1, II-12; patient 2, II-10; and a non-demented 68 year old member of the family, III.1, who is a son of the eldest affected brother (II.1). Mutation screening in APP exons 16 and 17, which code the Abeta peptide sequence and the surrounding region,

Abbreviations: Abeta, amyloid β peptide; APP, amyloid precursor protein; FAD, familial Alzheimer's disease; PCR-SSCP, polymerase chain reaction single strand conformational polymorphism

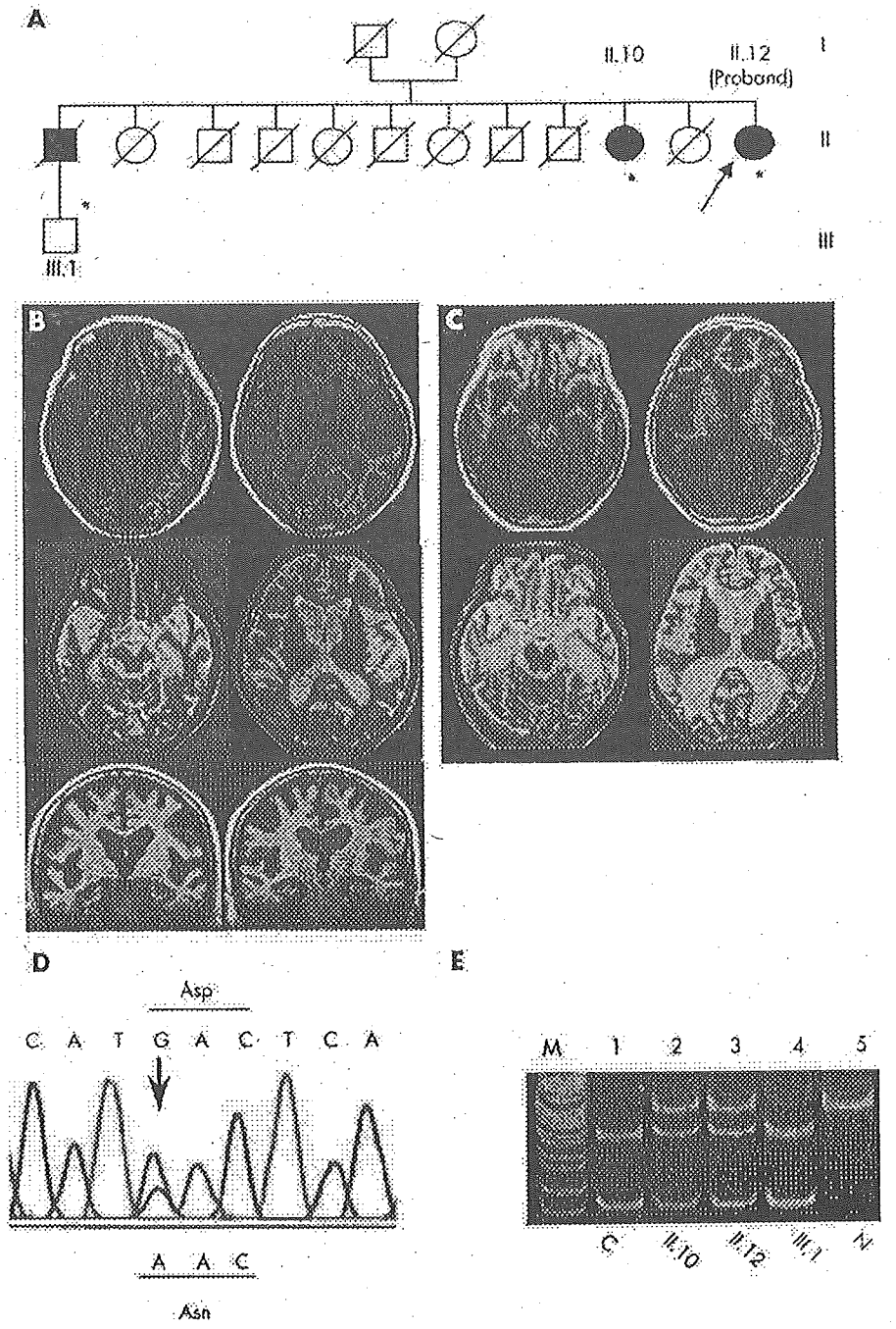

Figure 1 Family tree, neuroimaging findings, and molecular analysis of the Japanese-Tottori pedigree. (A) The pedigree: the proband is indicated by an arrow. Samples (*) were taken from three individuals for DNA analysis. (B) Axial and coronal magnetic resonance images (MRI) of the proband (II.12) at age 71 showing diffuse and severe cortical atrophy predominantly in the internal portion of the temporal lobes, without focal cerebral infarction or haemorrhage. (C) Axial MRI of the elder sister at age 71 showing marked cortical atrophy with mild periventricular hyperintensity lesions in T2 weighted images. (D) Direct sequencing of APP exon 16 PCR product derived from patient 1 (II.12) demonstrates heterozygosity for GAC-to-AAC nucleotide substitution in codon 678 of APP gene (APP770 numbering), that corresponds to an aspartic acid (Asp) to an asparagine residue (Asn) (D678N). (E) Characterisation of the D678N missense mutation in the APP gene. PCR-SSCP analysis of APP exon 16 showed extraconformers in patient 1 (II.12; lane 2) but not in controls (C) (lanes 1, 3, and 4). *Hinf*I RFLP analysis reveals that only the affected members (II.10; lanes 2 and II.12; lane 3) are heterozygous for D678N. C, a 68 year old non-demented family member (III.1; lane 1); N, undigested PCR product (lane 5).

were carried out by polymerase chain reaction single strand conformational polymorphism (PCR-SSCP) analysis. The primer sequences are as follows: APP exon16, APP Ex16Fw; 5'-TAGAAAGAAGTTTTGGGTAGGCTTT-3' and APP Ex16-Rv; 5'-AGAGTTAATAGGTCATTTGGCAAGACA-3', APP exon17, APP Ex17-Fw; 5'-AATGAAATTCTTCTAATTGCGTTT-3' and APP Ex17-Rv; 5'-TTCTCTCATAGTCTTAATTCCCACTT-3'. PCR was done using a Hot Start PCR kit (TaKaRa, Tokyo, Japan), following the manufacturer's instructions. For SSCP analysis, the PCR products were denatured in formamide containing buffer, and electrophoresed on 12% acrylamide gels with 10% glycerol at 4°C for 20 hours at 200 V, followed by visualisation using silver staining. The PCR product showing abnormal bands on SSCP analysis, predicting the presence of a mutant allele, was purified and sequenced. Sequence analyses of PS1 and PS2 coding regions using the RT-PCR product from

cultured skin fibroblasts of patients 1 and 2 were also undertaken. The *APOE* genotype was determined by a standard PCR *HhaI* restriction enzyme digestion assay.[11]

RESULTS

PCR-SSCP analysis of APP exon 16 showed abnormal bands in the proband sample (data not shown). Sequence analysis of the APP exon 16 revealed a GAC to AAC nucleotide substitution in codon 678 of the APP gene that causes an amino acid substitution of Asp to Asn (D678N) (fig 1D). No pathogenic mutations in APP exon 17 were detected in the proband sample (data not shown). Because D678N mutation abolishes the *Hinf* I restriction site, we looked for the presence of this mutation by *Hinf* I restriction fragment length polymorphism (RFLP) analysis of genomic amplicons. Analysis of the samples from the two affected members (patients 1 and 2) and a 68 year old unaffected member showed that only the affected members were heterozygous for the D678N mutation (fig 1E). The *Hinf* I RFLP analysis also showed the absence of this mutation in samples from 215 patients with sporadic Alzheimer's disease, five members of another five independent early onset FAD pedigrees, and 102 age matched control subjects. These data indicate that the D678N mutation is specific to the Jp-T pedigree. In addition, all members of this pedigree tested had the epsilon3/epsilon3 genotype of the *APOE* gene. Sequence analyses of PS 1 and PS2 coding regions using the reverse transcriptase polymerase chain reaction products from cultured skin fibroblasts of patients 1 and 2 failed to identify a pathogenic mutation (data not shown).

DISCUSSION

We report a novel D678N mutation in the APP gene identified in a Japanese FAD pedigree. This mutation causes an amino acid substitution of Asp at position 7 of Abeta (Asp7-Abeta) with Asn (Asn7-Abeta). The clinical manifestations of the mutations located close to the β- and γ-secretase cleavage sites—which directly affect the proteolytic processing of APP—are those of typical early onset FAD.[1 12–21] In contrast, the mutations causing amino acid substitutions within internal sequence of Abeta represent variable clinical and pathological features, presenting with different degrees of amyloid angiopathy or Alzheimer's disease pathology—for example, hereditary amyloid angiopathy with cerebral haemorrhage (E693Q and E693K),[4] Alzheimer's disease with repeated stroke (A692G),[5] dementia with severe amyloid angiopathy (D694N), or Alzheimer's disease without stroke (E693G).[6 7] Although the pathological features of our D678N mutant patients are still unknown, their clinical manifestations were those of typical Alzheimer's disease, characterised by progressive dementia without cerebrovascular complications in the clinical history and neuroradiological examination, fulfilling the NINCDS-ADRDA criteria for Alzheimer's disease.

A recent report described the identification of novel APP H677R mutation adjacent to the D678N mutation, which causes an amino acid substitution of His at position 6 of Abeta (His6-Abeta) with Arg (Arg6-Abeta).[22] Because the H677R mutation was present in one of two siblings with pathologically proven Alzheimer's disease in this report (mean age at onset 55 years), the pathogenicity of this intra-Abeta sequence variant needs to be clarified. However, it is assumed that the APP D678N and H677R mutations have a substantial impact on the pathomechanism of the development of FAD.

Currently we are studying the APP metabolism in cells transfected with D678N APP, as well as carrying out in vitro fibrillisation assays using synthetic Asn7-Abeta, but we have not seen increased production of Abeta (including Abeta42), or acceleration of Abeta aggregation (Hashimoto T, Saido T C, Iwatsubo T, unpublished observations). Alternatively, it has been reported that Asp residues of Abeta peptide (Asp1, Asp7, and Asp23) play an important role not only in fibril production but also in protofibril formation and initial α-helix formation. Artificial replacement from Asp residue to Asn residue at position 7 of Abeta has been reported to show altered fibrillogenesis kinetics (delayed helix formation) relative to wild type Abeta peptide.[23] Another study revealed that synthetic Asn7-Abeta peptide has the reduced ability to activate the classical complement pathway activation compared with wild type Abeta, the implications of which may also be relevant to the pathogenesis of Alzheimer's disease caused by this mutation.[24]

From these results, we postulate that D678N may be a novel type of APP mutation linked to FAD, which may cause Alzheimer's disease by an as yet uncharacterised mechanism—for example, alteration in fibrillogenic or catabolic properties of mutated Abeta or misfunction of APP, eventually leading to Alzheimer's disease. We await further structural modelling and Abeta metabolism studies, or in vitro models such as animals that are transgenic for this novel mutation. 3

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A haplotype of the methylenetetrahydrofolate reductase gene is protective against late-onset Alzheimer's disease

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Abstract

Epidemiological studies have shown that elevated plasma homocysteine (Hcy) levels play an important role in the pathogenesis of Alzheimer's disease (AD). In spite of the evidence that a C677T polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene elevates plasma Hcy levels, the impact of the C677T polymorphism on the development of AD is controversial. Here, we performed a genetic case-control study in a Japanese population to investigate whether three polymorphisms of the MTHFR gene, C677T (Ala222Val), A1298C (Glu429Ala), and A1793G (Arg594Gln), are associated with the development of late-onset AD (LOAD). In our study, the MTHFR gene had four major regional haplotypes: Haplotype A (677C-1298A-1793G), Haplotype B (677T-1298A-1793G), Haplotype C (677C-1298C-1793G), and Haplotype D (677C-1298C-1793A). The frequency of Haplotype C in LOAD was significantly lower than that in control group. Furthermore, the benefit conferred by the presence of at least one Haplotype C was stronger in LOAD patients who lacked the ApoE ε4 allele (OR = 0.293; 95% CI = 0.115–0.744; $P = 0.010$). The results indicate that Haplotype C of the MTHFR gene is protective against the development of LOAD.

Keywords: MTHFR gene; Haplotype; C677T; A1298C; A1793G; Association; Protective; Alzheimer

1. Introduction

Alzheimer's disease (AD) is one of the major neurodegenerative diseases in elderly population. Recent epidemiological studies have demonstrated that elevated levels of plasma homocysteine (Hcy) may play an important role in the pathogenesis of AD [3,19]. However, the detailed pathomechanism by which elevated plasma Hcy levels finally lead to AD is still uncertain.

Methylenetetrahydrofolate reductase (MTHFR; MIM *607093, EC 1.5.1.20) is one of the central enzymes for DNA synthesis and Hcy metabolism. In spite of the evidence that the C677T TT genotype (or the T allele) increases Hcy levels (particular in folate deficiency state) [8], its impact on the development of AD has been controversial [2,13,15]. No association studies of a second common polymorphism, A1298C (Glu429Ala) with AD were conducted.

Recent full genome scan studies have demonstrated the multiple candidate loci for late-onset AD (LOAD) including chromosome 1 [9,12,14]. The MTHFR gene locates chromosome 1p36.3 [5] and is predicted to be susceptible to LOAD. Our study was designed to evaluate whether the polymorphisms or the combined haplotypes of the MTHFR gene have an impact on the development of LOAD. We examined three MTHFR gene polymorphisms, C677T (Ala222Val), A1298C (Glu429Ala), and A1793G (Arg594Gln) (NCBI dbSNP cluster ID: rs2066462, rs1801131, and rs2274976, respectively), and the regional haplotypes derived from the three polymorphisms in a LOAD group and a control group.

2. Materials and methods

The study enrolled subjects from a western region of Japan. The diagnosis of AD was determined clinically according to the DSM-III-R and NINCDS-ADRDA criteria. Age at onset was defined by the appearance of the first clinical symptoms.

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